AD_____

Award Number: W81XWH-05-1-0255

TITLE: Specific Reversible Cytostatic Protection of Normal Cells Against Chemotherapeutics in Breast Cancer Therapy

PRINCIPAL INVESTIGATOR: Benjamin B Mull

CONTRACTING ORGANIZATION: The University of Texas
M.D. Anderson Cancer Center
Houston, TX 77030

REPORT DATE: March 2006

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01-03-2006	Annual Summary	21 Feb 2005 – 20 Feb 2006

4. TITLE AND SUBTITLE

Specific Reversible Cytostatic Protection of Normal Cells Against Chemotherapeutics in Breast Cancer Therapy

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-05-1-0255

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Benjamin B Mull

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The University of Texas
M.D. Anderson Cancer Center
Houston, TX 77030

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES
Original contains colored plates: ALL DTIC reproductions will be in black and white.

14. ABSTRACT

The adverse effects of cancer chemotherapy are widely recognized. Hair loss,gastrointestinal discomfort, lethargy and anorexia are quite common. The cause for these events is the nonspecific nature of current cancer treatment agents. Cytotoxic drugs, while effective at killing proliferating tumor cells, also target normal dividing cells. It is the purpose of this study to develop a proven in vitro strategy to protect normal dividing cells using a cytostatic agent, UCN-01. The reversible arrest of normally dividing tissues in mice will be examined for improved tolerance of chemotherapeutics. This protective effect will also be evaluated in mice bearing orthotopically implanted breast tumors.

15. SUBJECT TERMS
Breast cancer, UCN-01, protection, cytostatic

16. SECURITY CLASSIFICATION OF:				18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE		9	19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU		

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

Introduction:

The side effects of cancer chemotherapy are well known. The purpose of this study is to determine if a protective protocol developed in cultured cells will be effective in an animal model. A cytostatic drug currently in preclinical trials, UCN-01, has been demonstrated to arrest normal but not tumors cells reversibly. Temporarily arrested cells can evade many of the toxicities of chemotherapeutic agents. The proposal reported upon here is to determine if UCN-01 can be used in an animal model to protect the normal dividing cells of the body from these toxic effects by placing them in a reversible state of arrest. The work to date has focused on the ability of the drug to arrest the tissues and the parameters of recovery from that arrest.

Body:

The first aim of this project is to find a working concentration of arresting agent (UCN-01) in nude mice. An optimal concentration would arrest the dividing cells of the small bowel without causing serious detrimental effects. As this is a novel study, both drug amounts and delivery methods need to be evaluated for effectiveness. The first experiment was to inject mice with 0-10 mg/kg UCN-01 intraperitoneally. Mice were sacrificed 48 hours post-injection and small bowel tissue was harvested and fixed. Flow analysis of bromodeoxyuridine (BrdU) incorporation showed no significant difference in the cell cycle kinetics of mice receiving drug versus controls. Because the previous *in vitro* studies showed an arresting effect at very low concentrations of UCN-01, we decided to switch the delivery method rather than escalate the dose. The next experiment was carried out by injecting the drug intramuscularly into the mice (hindleg). Mice received vehicle control (DMSO), 0.63 mg/kg or 10 mg/kg UCN-01. The results indicated a slowdown of the cell cycle in the small bowel tissue. As seen in Figure 1, mice receiving DMSO had a much higher percentage of fluorescent divided labeled cells (% f^{ld}) compared to mice receiving the drug. Fluorescent labeled divided cells are those which have incorporated the BrdU in S phase and have subsequently completed mitosis. A decrease in this value indicates an arrest or slowdown in the progression through the cell cycle. The 0.63 mg/kg cohort and the 10 mg/kg cohort both demonstrated a decrease in f^{ld}. This experiment was repeated to verify the results. In addition, IHC was performed on fixed sections of gut tissue to visually verify that the cells of the crypt lining the intestine walls were arrested by the drug treatment. It was also noted in the initial experiments that untreated mice (PBS controls) have much greater variability in their cell cycle kinetics than treated mice (DMSO or UCN-01). It was decided that a small group of mice would be given PBS or mock injections and then analyzed for cell cycle kinetics. This would give us a "baseline" values from which to compare the subsequent treated groups. After analysis, the f^{ld} values averaged to 0.99%. This work has addressed the ability of UCN-01 to arrest the dividing cells of the gut, as well as refined a system to measure the kinetics of these cells.

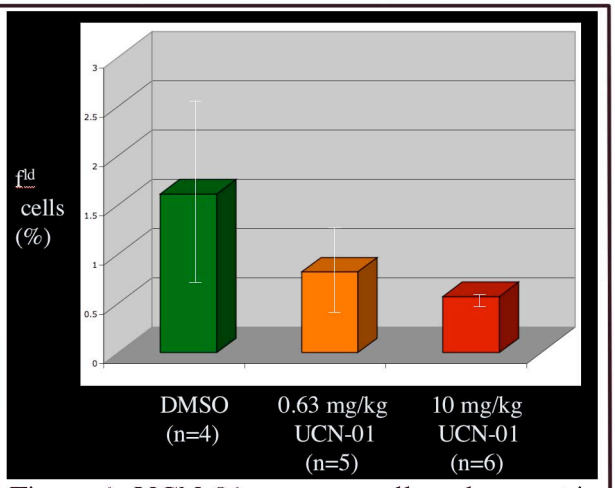

Figure 1: UCN-01 causes a cell cycle arrest in small bowel epithelium.

The second aim of this project is to demonstrate that the cell cycle arrest via UCN-01 is reversible. If this effect were permanent, no improved tolerance to chemotherapeutics would be realized. The previous experiments indicated that a significant arrest could be achieved by treating with 0.63-10 mg/kg UCN-01. An intermediate value, 5 mg/kg, was chosen for these experiments. 3 cohorts of 10 mice each were treated with 5 mg/kg

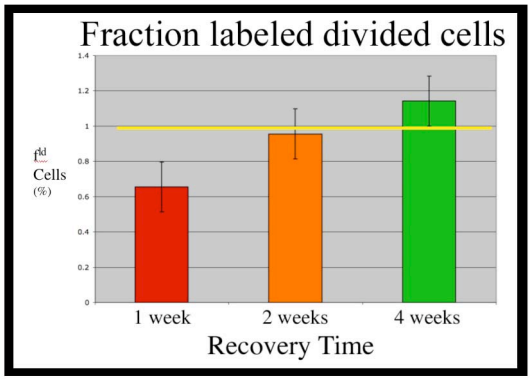

Figure 2: Arrest due to UCN-01 treatment abates between 2 and 4 weeks. Yellow line is average mouse value of 0.99% f^{ld}.

UCN-01, and then evaluated at either 1, 2 or 4 weeks post-injection to determine when and if the arresting effect of the drug abated. As seen in Figure 2, the f^{ld} percentage of the treated mice approached the normal average value by 2 weeks, and actually becomes slightly hyperproliferative by week 4. This last value is probably a result of the tissue trying to remodel after the prolonged decrease in cell proliferation. This experiment was repeated (3 cohorts, 10 mice each) to verify results. It was also noted that the DMSO controls from specific aim 1 were higher than the average value for untreated mice. To address this question, 30 mice were injected with the DMSO volume equivalent of UCN-01 at 5mg/kg. 10 mice were sacrificed at 1, 2 and 4 weeks and gut tissue was analyzed for cell cycle kinetics. As is seen in figure 3, the small bowel cells actually increased in their proliferation following DMSO treatment, and even by 4 weeks had not completely returned to a normal proliferative state. This indicates that the vehicle being used for our drug treatment actually has an antagonistic effect. Further experiments will use a more concentrated UCN-01 solution to minimize this effect as much as possible. This work has demonstrated that the arrest via UCN-01 is reversible, and the time course for this dose range appears to be between two and four weeks post-treament.

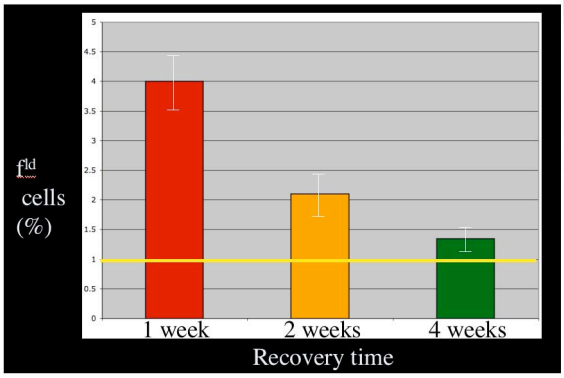

Figure 3: DMSO treatment has a UCN-01 antagonistic effect. Yellow line is average mouse f^{ld} value of 0.99%

Key research accomplishments:

- Determined an optimal method of injection for arresting agent (UCN-01 intramuscularly)
- Determined a working UCN-01 dose range for effective arrest of dividing cells of the small bowel (0.63 – 10 mg/kg)
- Determined an average cell cycle kinetic parameter for normal mice (0.99% f^{ld})
- Determined the time recovery time required for reversal of the arresting effect of UCN-01 (2 weeks)

Reportable outcomes:

No reportable outcomes as of this writing.

Conclusions:

UCN-01 can effectively arrest the normal dividing cells of the small bowel at a low working concentration. This effect wears off between 2 and 4 weeks post-treatment. The carrier for UCN-01, DMSO, antagonizes its effectiveness. Future experiments will need to use a more concentrated drug solution to minimize the interference of DMSO.